

Loss of genetic material within 1p and 19q chromosomal arms in low grade gliomas of central nervous system

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Abstract

Diffuse gliomas can constitute up to one third of all gliomas diagnosed in neurosurgical centers. Their invasive growth, progression to more malignant lesions, and the lack of standardized management guidelines render a significant clinical problem. The discovery of 1p and 19q chromosomal arms deletion in neoplastic cells will probably influence both more objective diagnosis and more accurate prediction of chemotherapy response. Defining the above mentioned deletion is becoming a standard procedure in Western European countries and in the USA when LGG is diagnosed. As a result an attempt has been made to detect deletion using fluorescence in situ hybridization and to determine its prognostic value.

Genetic material from 34 grade II gliomas was examined. Separate 1p and 19q deletions were discovered in 14 and 16 cases respectively. Simultaneous occurrence of both was observed in 12. The frequency of occurrence of simultaneous deletions 1p and 19q varied based on histopathological diagnosis. This disorder was not observed in astrocytomas, in oligoastrocytomas it appeared in 50% cases. The highest incidence of deletion was noted in oligodendrogliomas and amounted to 66.7%, $p < 0.005$. Median survival in patients with diagnosed 1p and 19q deletion in their neoplastic cells is twice longer in comparison with patients in whom no such deletion was observed (80 months vs. 41 months, $p < 0.05$). Frontal location of a tumor occurred to be a statistically significant factor unfavorable for prognosis, $p < 0.05$. In the work presented the fluorescence in situ hybridization was successfully applied to identify deletion 1p/19q. Its incidence depends on the type of diagnosed glioma. Deletions also have prognostic significance in the test group what constitutes the basis for inclusion of determining deletion 1p/19q into diagnostic and treatment algorithm in LGGs.

Key words: astrocytoma, oligodendroglioma, oligoastrocytoma, low grade gliomas, 1p, 19q.

Introduction

The definition of low grade glioma encompasses three types of WHO grade II tumours including: diffuse astrocytoma (DA), oligodendroglioma (OD), and mixed glioma – oligoastrocytoma (OA). They constitute up to

30% of all glial tumours of the central nervous system. Distinguishing between these three types may be challenging due to both subjectivity of pathologists' judgment and lack of reliable objective markers confirming the diagnosis. The WHO classification does not provide the exact cut-off values of the oligodendroglial

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and astrocytic component between three aforementioned types of tumours. Thus, discrepancy among pathologists exists, some use a 50% threshold level for the oligodendroglial component in making the diagnosis of oligodendroglioma [31], whereas others use a 10% cut-off [15].

Recent publications suggest that deletion of chromosomal arm 1p/19q may play such an indicative role in the diagnosis of low grade gliomas (LGG) [1,11]. LGGs demonstrate a broad range of genetic disturbances. One of the frequent alterations is simultaneous deletion of the short arm of chromosome 1 and the long arm of chromosome 19 [9]. Although chromosome 19q deletion used to be regarded a feature of both oligodendrogliomas and astrocytomas, further studies have shown its predominance in the former group [28]. Both deletions of 1p/19q frequently occur together and are seldom found as a separate alteration [4,19,24]. It is estimated that frequency of concurrent 1p and 19q deletion approaches 70-80% in oligodendrogliomas, 30-50% in mixed gliomas and only 7-15% in astrocytomas [4,19, 24]. The deletion of 1p/19q is referred to as the most common genetic abnormality observed in oligodendrogliomas. Therefore, 1p/19q deletion seems to be a reliable signature of this neoplasm.

In addition, the deletion of 1p/19q is additionally a predictive factor in OD. Cairncross *et al.* demonstrated that 1p deletion is a marker of the tumour chemosensitivity to procarbazine, lomustine and vincristine scheme (PCV) and concurrent 1p/19q deletion is a positive factor for a longer time to progression in anaplastic oligodendrogliomas [7]. Additionally, the use of multivariate statistics showed that survival in patients with 1p/19q loss is statistically longer than in patients without this alteration. These results were confirmed by retrospective analyses based on clinical studies EORTC 26951 and RTOG 9402. Both proved that presence of co-deletion correlates with the extended time to progression and time to death in grade III [3,6] and grade II gliomas [12]. Moreover, the latter study disclosed beneficial response to chemotherapy in patients with LOH 1p/19q.

Based on these data we performed this study at the Medical University of Gdansk in order to correlate the status of 1p/19q deletion in LGGs with tumour morphology and patients' demographic factors.

Material and methods

We retrieved tissue samples of WHO grade II gliomas from the Department of Neurosurgery and

the Department of Pathology over a period from 1997 to 2002. The tissue available for study was obtained in 68 cases. Verified diagnosis was based on the 2007 WHO classification of CNS tumours (Fig. 1). LGG diagnosis was confirmed in 47 cases. Due to the small amount of tissue, neoplastic material from 39 cases was prepared for tissue microarray study (TMA) and following fluorescence in situ hybridization (FISH). During the revision process most representative parts of each tumour were marked.

Selected tissue was taken into TMA acceptor blocks using tissue arrayer (MTI, Beecher Instruments, Silver Spring, MD) with 1.5 mm punches. TMAs were cut into 4-micrometer slices. One slice from each array was stained H&E for histological control.

Fluorescence in situ hybridization was performed using commercially available LSI 1p36/1q25 and LSI 19q13/19p13 Dual Color probes (Abbott Laboratories,

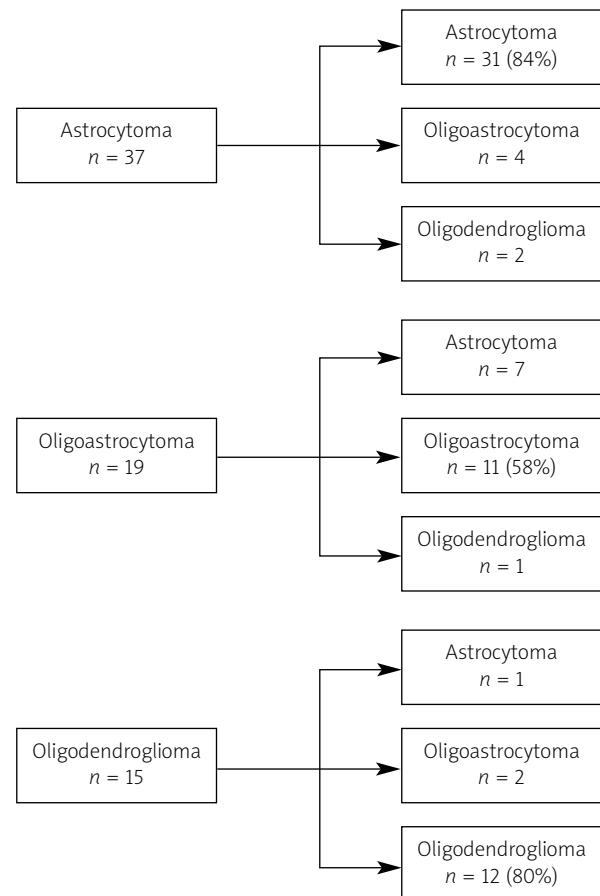


Fig. 1. Changes of primary (left) diagnoses after revision (right) and concordance percentage among the three LGG types.

previously Vysis). The kit contains two mixtures. The first one consists of probe for locus 1p36 and control probe for 1q25. The second mixture stains an examined locus 19q13 (red) and a control locus 19p13 (green). Four signals are observed in normal diploid nuclei – two red and two green ones. In 34 patients FISH proved sufficient to supply reliable results.

Signals from 50 non-overlapping nuclei were assessed and to eliminate random sectioned nuclei, deletion was diagnosed when more than 20% of examined signals in relation to control signals were absent [13]. In all borderline cases (with the absence of 15-25% of signals), additional 50 nuclei were reassessed.

The analysed group of 34 patients with LGG consisted of 21 (62%) males and 13 (38%) females. Average age at the time of operation was 40.9 years (range: 18.2-62.8; SD = 10.8). In most cases, the tumour tissue was obtained by resection. Only in 4 (11.7%) cases, tissue from stereotactic biopsy was acquired. The histological diagnoses were as follows: diffuse astrocytoma, grade II (AII; $n = 12$, 35%), oligoastrocytoma (OAI, $n = 10$; 30%), oligodendroglioma (OII, $n = 12$; 35%).

Survival time was defined as time from the date of the operation to death or, in censored cases, time to exclusion of the patient death. All appropriate data were imported to Statistica (Statsoft) – statistical analysis software. Median survival times calculation and survival graphs were performed using Kaplan-Meier analysis. Impact of prognostic factors was assessed by univariate log-rank tests. In order to identify independent factors affecting survival, statistically significant factors were introduced in multivariate analysis (Cox proportional hazard model). All results were considered statistically significant when $p < 0.05$.

Results

Fluorescence in situ hybridization revealed 1p deletion in 14 (41%) cases and 19q deletion in 16 cases (47%, see Table I). Simultaneous deletion of 1p/19q was

detected in 12 cases (correlation coefficient $F^2 = 0.65$, $p < 0.0002$).

Codeletion of 1p/19q correlated strongly with the histological type. It was not identified in AII, however, it amounted to 50% in OAI and to 66.7% in OII ($p = 0.003$, Table I). The anatomical location of the tumour had some dependence on the presence of this alteration as only one in nine tumours located in the temporal lobe bore a genetic defect, while half of frontal tumours presented 1p/19q deletion (8/16, P value for χ^2 test equals 0.051).

Median time of follow-up was 65 months. Median time of patients' survival was 69.1 months (Fig. 2A). At the last follow-up, 10 patients were alive (29.4%). Median time of survival differed among tumour types from 25 months in AII, through 53.2 months in OAI, to 85.5 months in OII ($p < 0.04$, Fig. 2B).

Simultaneous deletion of 1p/19q correlated with longer survival. Median survival for patients with and without this alteration was 80 and 41 months, respectively ($p < 0.047$) (Fig. 2C).

Among other demographic factors (sex, age at diagnosis) and clinical factors (affected hemisphere, frontal vs. other localization, adjuvant therapies), the only one having negative influence on the survival was frontal localization of the tumour ($p < 0.046$, log-rank test) (Fig. 2D).

Therefore, two factors were chosen for the multivariate analysis – histological type and 1p/19q deletion. Lack of 1p/19q deletion correlated with an increased risk of death to a level of 2.855 (CI: 1.040-7.839). Other factors did not correlate with time of survival.

Discussion

Loss of 1p and 19q chromosomal arms is one of the most important molecular signatures in the diagnosis of central nervous system tumours. It is quite specific to oligodendroglial tumours and due to lack of any other phenotype and genetic marker typical of these neoplasms it becomes of interest for pathologists.

Table I. Detailed information regarding loss of genetic material on chromosomal arms 1p and 19q with regard to coexistence of these defects

	1p deletion	19q deletion	1p/19 codeletion	No defect
Astrocytoma	2	2	0	8
Oligoastrocytoma	4	6	4	4
Oligodendroglioma	8	8	8	4

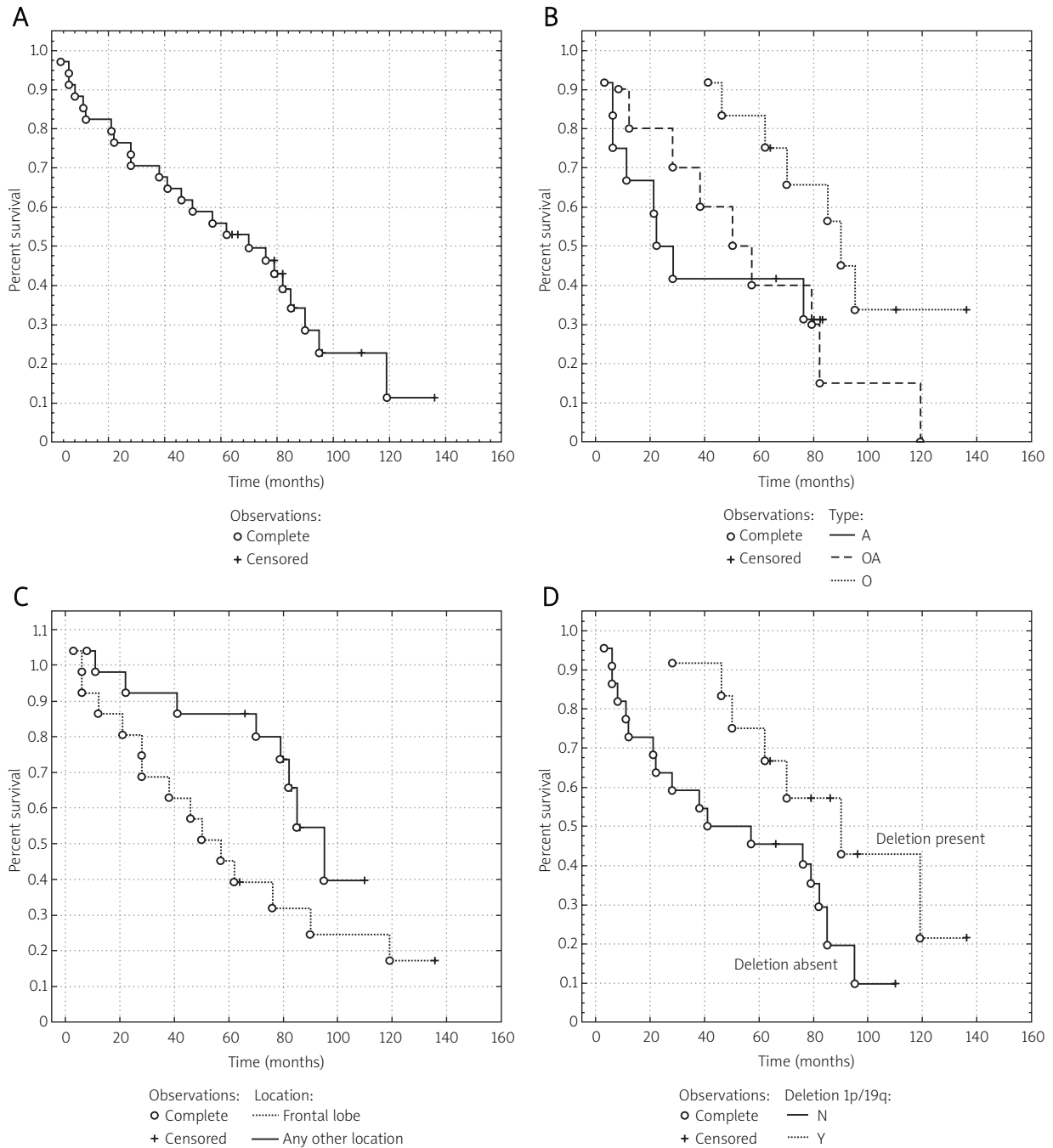


Fig. 2. Kaplan-Meier graphs depicting survivals of the whole examined group **(A)** and divided into subgroups. **B)** Survival influenced by histological type of tumour (A – astrocytoma, AO – oligoastrocytoma, O – oligodendroglioma). **C)** Comparison of two groups: one with tumour localized in the frontal lobe, second with different localization. The first group shows shorter survival, log-rank test, $p < 0.05$. **D)** Group with loss of chromosomal arms 1p/19q as opposed to a group with unchanged chromosomes. The latter has shorter survival, confirmed by log-rank test, $p < 0.05$.

As the morphological features typical of neoplasms with oligodendroglial differentiation occur only in a subset of these tumours and they depend on the tissue processing, the diagnosis of these tumours is always a challenge. Loss of 1p/19q seems to preferentially occur in tumours sharing these specific morphological features of oligodendroglial tumours (monomorphous cell population with 'fried egg' appearance, roundish nuclei). However, this classical histological presentation is present in a portion of oligodendroglial tumours. In others, the pathological diagnosis relies to a great extent on the subjective interpretation of a pathologist. Therefore, the diagnostic discrepancy is a true nightmare in the diagnosis of these neoplasms. Additionally, finding of the morphological features of oligodendroglial-like cells requires not only differentiation with tumours of astrocytic lineage but also other neoplasms having this phenotype (dysembryoplastic neuroepithelial tumour, neurocytoma) [1]. As determination of 1p/19q deletion presents also a prognostic and predictive value, analysis of this alteration is not of diagnostic importance but has therapeutic consequences. Therefore, we undertook this study to reveal its incidence in a group of diffuse LGGs.

The frequency of LGGs among all glial tumours we found was rather high as it amounted to 32.8%. This value is in accordance with incidence [16]. Age at diagnosis is typical of this group of neoplasms and concordant with other published data [23].

There is no consensus with regard to the threshold of the oligodendroglial and astrocytic component required to diagnose mixed glioma. The WHO classification does not provide strict guidelines, either. This may constitute one of the causes of high differences in the number of oligoastrocytoma reported by other authors [22-24]. It can be explained by the fact that LGGs form a spectrum of pathological images, as presented by Ueki [32]. He proposed considering LGGs as a continuous spectrum of proliferative diseases occupied by astrocytoma and oligodendroglioma at two opposite ends and mixed glioma occupying its central part. Histological features do not make it possible to place a sharp border between described entities. An additional support to this thesis brings identification of *IDH2* mutation shared by diffuse astrocytomas and oligodendrogliomas and regarded as an early molecular alteration in LGGs [21].

Analysis of both cellular components in mixed gliomas using microdissection, splitting astrocytic and oligo-

dendroglial cell lines, provides evidence that both lines bear the same genetic defects. Seemingly, two subgroups may be identified among mixed gliomas [18]. One shows typical genotype for oligodendroglioma and the other – for astrocytoma [18,26]. Most importantly, both subgroups are indistinguishable on pathological examination. Therefore, more objective and precise markers are desirable to provide an optimal therapeutic approach to patients with these tumours.

As we showed and this observation supports earlier publications, deletions of chromosomal arms 1p and 19q are mutually strongly correlated [23,24]. We found simultaneous deletion of both chromosomal regions in 12/14 cases (85.7%) with 1p deletion and 12/16 cases (75%) with 19q deletion. Rarely, do these defects occur as independent alterations. Exclusive 19q deletion was found in diffuse astrocytomas and oligoastrocytomas and this is consistent with the results of previous surveys. Similarly, isolated deletion of 1p, if present in LGGs, was related to "pure" astrocytomas. We have not detected isolated deletions of either of these loci – all oligodendrogliomas presented with concurrent deletion of 1p and 19q.

Since not all oligodendrogliomas demonstrate 1p/19q deletion, morphological features that could help differentiate these two groups were deeply sought. In a study by Burger *et al.*, 18 cases of LGGs were revised [5]. In each case when primary and revised diagnosis uniformly showed an oligodendroglial type, 1p/19q deletion was found. On the other hand, when both diagnoses were inconsistent and astrocytoma was diagnosed during revision – this alteration was not identified. We presented very similar results. Therefore, we may conclude that when the tumour presents unequivocal morphological features of oligodendroglioma, 1p/19q deletion may be suspected. Additional evidence is presented by Sasaki *et al.* [29]. In their paper published in 2002, oligodendrogliomas were divided into two groups – one including tumours of classic oligodendroglial image and the other having some astrocytoma features. Deletion of 1p/19q was detected in 86% of tumours from the former group and 27% – in the latter group.

Among presumed predictive factors, three turned out to be statistically significant: histological type, 1p/19q deletion and localization (frontal vs. other). These results are in concordance with literature data, which confirm strong influence of the first two factors on survival time [6,8,12,25].

We proved that simultaneous 1p/19q deletion is a strong factor for better prognosis. If it occurs, the difference in median survival is 80 months in contrast to patients whose tumours do not present this alteration (41 months). This difference is independent of the histological type. These results justify genetic testing not only for oligodendrogliomas but for the whole group of LGGs.

Similarly to the other studies, we have noticed a correlation between 1p/19q loss and location of the tumour. In addition to former reports that tumours with temporal, insular and diencephalic location usually correlated with the preservation of 1p/19q chromosomal arms [33], we have found that tumours developing in the frontal lobe was of a much higher incidence of 1p/19q loss.

Likewise, LGGs have age-dependent differences in occurrence of 1p/19q deletion. Although histologically identical, WHO grade II diffuse gliomas developing in childhood usually do not show this alteration [14,27]. Due to the low incidence of LGGs in children, it is hard to evaluate influence of this genetic abnormality on survival. In our two youngest patients (case 40 aged 18 diagnosed with astrocytoma; case 60 aged 21 diagnosed with oligodendroglioma), this genetic defect was not found. Seemingly, childhood LGGs present other types of glial proliferation despite the similar histological presentation. Molecular alterations play an important role in this regard.

Other factors analysed in our group of patients did not correlate with survival time. It confirms the former publications, excluding age at diagnosis. Most of the authors noticed shorter survival in patients aged over 40. This observation probably did not result from differences in the treatment [25]. There are three possible explanations for this phenomenon. One is the clinical status of an elder patient who may suffer from comorbidities. Secondly, these patients usually present with more advanced neoplasms at the time of diagnosis, that directly results in increased tumour volume [2]. Moreover, aggressiveness of the gliomas developing in younger patients is seemingly lower. This is supported by a shorter time to progression [30], and lower proliferative capacity of the tumours in this age group of patients [10,20].

Concluding, detection of 1p/19q deletion in LGGs has two potential benefits. Firstly, due to its high incidence in oligodendrogliomas, it has a huge value as an additional tool in their correct and objective diag-

nosis. As a consequence, this may be the first step for introduction of molecularly-based classification of glial tumours [17]. Secondly, this codeletion is a factor determining longer survival. Inclusion of a patient in a specific prognostic group may influence intensity of conducted treatment. The above features constituted the basis to include genetic examination in all patients with probable LGG in other countries. Results of the presented paper support application of 1p/19q detection in common neurosurgical and pathological practice.

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